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Honeybees mark with scent and reject recently visited flowers

Martin Giurfa* and Josué A. Núñez

Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,
Pab. II – Ciudad Universitaria, CP 1428, Buenos Aires, Argentina

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Summary. Experimental evidence is reported for flower-marking by honeybees (*Apis mellifera ligustica*) while they were foraging on an artificial patch of flowers yielding a continuous and equal flow of sucrose solution. Honeybees marked with scent and rejected all recently visited and nectar-depleted flowers. The short fade-out time of this scent allowed discrimination of flowers that temporarily provided no food. The repellent nature of this scent mark was demonstrated by the use of an air extractor connected to the patch; when the apparatus was turned on, the rejection behaviour disappeared. The movement pattern of foraging bees also contributed to foraging efficiency, as the probability of an immediate return to the flower just abandoned was very low. However, when a quick repeat visit took place, the presence of the repellent scent-mark promoted rapid rejection.

Key words: Honeybee – Scent-mark – Movement pattern – Foraging behaviour – Foraging efficiency

Although it is most often assumed that nectar-feeding animals can assess nectar content of flowers only after probing, sometimes bees may distinguish full from empty flowers visually (Thorp et al. 1975), by the scent of nectar itself (Heinrich 1979; Marden 1984) or by intrafloral gradients of humidity (Corbet et al. 1979). Other discrimination cues often reported are pheromones employed by some bee species during foraging (Ribbands 1955; Lindauer and Kerr 1958; Frisch 1967; Free 1968; Butler et al. 1969; Free and Williams 1972; Ferguson and Free 1979; Cameron 1981; Free et al. 1982; Schmitt and Bertsch 1990). All the previously studied pheromones elicit the alighting of bees on the marked surfaces and can therefore be considered attraction pheromones. However, Núñez (1967) demonstrated with an artificial patch of flowers that honeybees employ a flower-marking scent that allows them to recognize and reject non-rewarding flowers, enabling them to focus their foraging activity on flowers with nectar supply. Although not demonstrated, the same conclusion was reached by other authors who have also observed bees approaching and avoiding non-rewarding flowers (Frankie and Vinson 1977; Corbet et al. 1984; Wetherwax 1986; Kato 1988). We report here new experimental evidence on the flower-marking strategy employed by honeybees while foraging on an artificial patch of flowers continuously supplied with the same sucrose flow. Our results indicate that honeybees mark with scent and reject all recently visited and depleted flowers and that both the repellent scent mark and foraging-movement patterns are important in foraging efficiency.

Materials and methods

We trained individually marked honeybee workers, *Apis mellifera ligustica*, from a hive located 50 m from the laboratory, to visit and collect 50% sucrose solution on a food source simulator (Núñez 1971) shown in Fig. 1. The apparatus consisted of an acrylic disc 50 cm in diameter with 12 identical, numbered, yellow flowers, spaced 10 cm from each other. Each flower received sucrose solution through a cannula connected to an automatic microburette of the Scholander type, driven by a synchromotor. The sugar flow provided was 2.04 µl/min (each flower received $\frac{1}{12}$ of this total flow, i.e. 0.17 µl/min). Previous calibrations were made in order to ensure that all the flowers were actually equally rewarding in terms of sucrose flow. Bees reached the sucrose solution through a PVC access tube on each flower. An air extractor was connected to the food source simulator as shown in Fig. 1. Each flower presented two perforations adjacent to the sugar cannula so that odour marks could be carried by activating the fan.

After pretraining, one bee was selected for measurements while the others were caged for later use. Each free-flying bee was observed over two visits. *Landings* were recorded in their temporal sequences, discriminating between *inspections* and *rejections*. Inspections were landings in which the bee fully entered the access tube of the artificial flowers and rejections were landings in which the bee introduced only the antennae into the access tube and immediately flew away. The *visit-time* (time elapsed from the bee's arrival at the food source simulator until its departure to the hive) and the *non-visit-time* (time spent away from the simulator between

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Present address and address for offprint requests: Institut für Tierphysiologie – Neurobiologie, Freie Universität Berlin, Königin-Luise-Str. 28–30, W-1000 Berlin 33, FRG

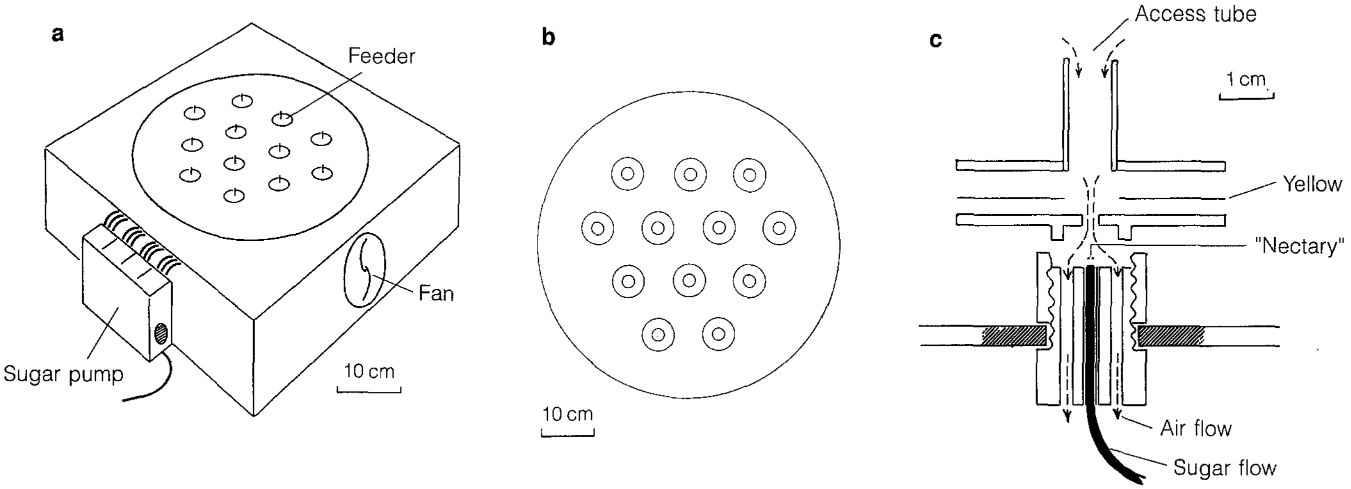

Fig. 1a–c. Food source simulator. **a** General view; **b** view from above of the artificial patch with 12 numbered flowers; **c** magnified detail of a flower

Table 1. Visit values of foraging honeybees at the food-source simulator under two experimental conditions (air extractor off and then on)

	Without air extractor (mean ± SE; $n=40$)	With air extractor (mean ± SE; $n=40$)	t	P
Inspections per visit	124.55 ± 5.07	169.83 ± 4.60	12.34	<0.001
Rejections per visit	11.43 ± 0.79	0.13 ± 0.05	14.24	<0.001
Flower visitation rate[1]	8.20 ± 0.18	8.19 ± 0.18	0.11	NS
Visit time (min)	16.59 ± 0.57	21.00 ± 0.63	8.96	<0.001

[1] Flower visitation rate is defined as the number of flower landings per minute

visits) were also recorded. In the second visit, after the air extractor had been activated, the same parameters were measured.

Only bees whose non-visit-times were less than 5 min were used (mean ± SE: 3.12 ± 0.13 min; $n=50$). Altogether 40 bees were studied, totalling 80 visits. In order to verify that treatment order did not affect results observed, 10 other bees were used as a control group with an inverted schedule (i.e. first visit with the fan on; second visit with the fan off).

The foraging movement pattern was analyzed for the original 40 bees. For this, we defined six categories of temporal foraging sequences: Event 0, defined as the immediate return to the flower just abandoned; Event 1, defined as the return to a particular flower after visiting one other; Event 2, defined as the return to a particular flower after visiting two different flowers, and so on until Event 5 (return to a particular flower after visiting five different flowers). By counting the number of such events in each bee visit, we established their probability of occurrence in the two experimental situations (with the air extractor off and on). We tested then whether all events presented the same probability of occurrence in both situations.

Furthermore, we established the percentage of rejections occurring in each event category with the air extractor off and on.

As in every case the same individual was measured twice, the paired-sample t test was used for statistical analysis (Zar 1984, 150–153). To avoid training to location, the artificial patch was sometimes irregularly rotated.

Results

Both the mean number of inspections per visit and the mean number of rejections per visit were strongly affected by whether the air extractor was on or off (Table 1). When the air extractor was on, bees made more flower-inspections ($t=12.34$, $df=39$, $P<0.001$) and performed only 0.13 ± 0.05 rejections per visit (mean ± SE, $n=40$). Previously, with the fan off, they performed 11.43 ± 0.79 rejections per visit ($n=40$; $t=14.24$, $P<0.001$). The flower visitation rate (flower landings/minute) of bees was the same with the fan off or on ($t=0.11$; NS). The length of the visit (visit-time) was significantly longer with the fan on ($t=8.96$, $P<0.001$); that is, visits to the patch were longer when bees made more flower inspections. The only source of variation in the experimental design was the type of treatment (fan on or off) and not the treatment sequence (Table 2). Each visit value from Table 1 was compared with the visit value from Table 2 obtained under a similar treatment. All eight comparisons were non-significant (two-sample t test).

The use of the air extractor did not affect the probability of occurrence of each event (Table 3). Paired comparisons were all non-significant (test for comparison of two proportions; Zar 1984, 395). However, comparison along a row shows that, in both experimental situations, the probability of occurrence was the same for Events 1, 2, 3, 4 and 5 ($\chi^2=8.95$, $df=4$, NS, with the air extractor off; $\chi^2=8.24$, $df=4$, NS, with the air extractor on) but was significantly lower for Event 0 ($\chi^2=265.25$, $df=5$, $P<0.001$ with the air extractor off; $\chi^2=247.31$, $df=5$,

Table 2. Visit values of foraging honeybees at the food-source simulator under the reversed sequence of conditions (air extractor on and then off)

	With air extractor (mean ± SE; $n=10$)	Without air extractor (mean ± SE; $n=10$)	t	P
Inspections per visit	162.90 ± 8.44	120.80 ± 6.12	8.51	<0.001
Rejections per visit	0.10 ± 0.10	9.30 ± 0.52	17.97	<0.001
Flower visitation rate	8.14 ± 0.19	8.17 ± 0.23	0.54	NS
Visit time (min)	20.27 ± 1.35	16.11 ± 1.02	6.73	<0.001

Table 3. Foraging visit Sequences: probability of occurrence of each event and percentage of rejections associated with experimental conditions A and B (air extractor off and air extractor on, respectively); $n=40$ bees

Event	0	1	2	3	4	5
P_A	0.06	0.20	0.18	0.18	0.18	0.21
P_B	0.06	0.20	0.17	0.19	0.19	0.20
P_A vs P_B	NS	NS	NS	NS	NS	NS
% A (% rejections in A)	89.99 ± 2.70	43.42 ± 2.51	8.25 ± 1.84	1.95 ± 0.60	0.16 ± 0.16	1.39 ± 1.39
% B (% rejections in B)	0.63 ± 0.44	0.59 ± 0.33	0.23 ± 0.23	0	0	0
t (%A vs %B)	31.63	16.40	4.28	3.22	1	1
P	< 0.001	< 0.001	<0.001	<0.005	NS	NS

P_A and P_B are the probabilities of occurrence of the events under experimental conditions A and B (air extractor off and air extractor on, respectively). % A and % B are the percentages of rejections evinced for the events under conditions A and B

$P<0.001$ with the air extractor on). In other words, the bees were less likely to revisit the flower just sampled, independently on the air extractor being turned off or on.

The percentage of rejections in each event category was established in both experimental situations (Table 3). The percentage of rejections was greatest (89.99 ± 2.70, mean ± SE, $n=40$) when bees returned to the flower just abandoned (Event 0) and decreased to values which are statistically undistinguishable from zero when bees visited four or more flowers before returning to the same one. With the air extractor, the percentage of rejections fell to zero in all six event categories (0.63 ± 0.44, 0.59 ± 0.33 and 0.23 ± 0.23, not significantly different from zero: $t=1.43$, 1.78, 1, respectively; all values NS). That is, immediate return to the flower just abandoned occurs with low probability, but when it takes place, the bees usually quickly abandon it.

Discussion

Results reported here are consistent with the existence of a repellent marking substance left by bees when leaving an inspected flower. This substance would be used not only to discriminate unproductive from rewarding flowers (Núñez 1967), but also in a broader context, when foraging on a patch where all flowers continuously provide nectar.

As previously said, the existence of such a repellent scent-mark was also suggested by Corbet et al. (1984) and Wetherwax (1986) in honeybees (*Apis mellifera*), by Frankie and Vinson (1977) in *Xylocopa virginica texana* and by Corbet et al. (1984) and Kato (1988) in bumblebees (*Bombus* sp.). In our work, the repellent nature of this scent-mark was demonstrated by the use of the air extractor, an experimental device that carried away the mark from the artificial flowers. When the extractor was activated, the level of inspections (i.e. the level of "acceptance" of the artificial flowers) increased. Had the scent-mark been attractive, bees would be predicted to reject all the flowers when the olfactory cue was absent, a result not observed in our experiments.

Foragers would benefit if they avoided recently depleted flowers until the nectar had been replenished and evaporation had attenuated the repellent scent mark. Our results indicate that this behaviour actually accurred and that in the Event 4 situation (6 flowers visited), with no air extraction, the scent-mark was no longer present (Table 3); with a flower visitation rate of 8 flowers/min (Tables 1 and 2), the endurance of the repellent scent could be estimated as 45 s or less. The rapid fading of the substance allows discrimination of flowers that temporarily provide no food. It is likely that a relatively volatile pheromone is used, but both the nature and the source of such pheromone are unknown.

Even if we demonstrated that bees which have depleted artificial flowers of sucrose syrup then proceed to mark them and deter further bee visits, other pheromone signals such as "footprints" (Butler et al. 1969) are used by honeybees to mark flowers as attractive sources of forage. Such attractant pheromones are long-term signals (Ferguson and Free 1979; Schmitt and Bertsch 1990) whereas the repellent scent-mark lasts only a short time.

Thus, the ultimate message given by both marks left on a flower will depend on the interaction between the attractive and repellent marks. Initially, after a flower has been visited and depleted of nectar, the repellent substance will promote its rapid rejection. Thereafter, as the mark volatilizes, the more persistent attractive signal will have a dominant influence and the flower will again be attractive to foragers, by which time the supply of nectar will be replenished.

The ability to recognize recently visited flowers allows bees to reduce the number of flower inspections necessary to attain the particular crop-load appropriate to this sugar flow in relation to distance from the hive (Núñez 1982). This strategy thus saves the time and energy required by a bee to crawl completely into the access tube and then to leave by backing out. Foraging times were consequently longer (Tables 1 and 2) if the scent-mark was removed from the artificial flowers by air-extraction. As pointed out by Kato (1988), a bee using such an olfactory cue would forage more efficiently than hypothetical random foragers.

As visit times were longer with the air extractor on, the negative scent-mark could also affect the balance between the two tendencies, *to stay* and *to go* at the food source, proposed to explain the foraging behaviour of bees (Núñez 1970). When the reward at the food source increases, the tendency to stay also increases until a threshold where the tendency of going back to the hive increases in turn. These two tendencies are assumed to be interdependent, and the presence of the negative mark could lower the threshold for abandoning the food source, reducing the actual time spent by the bee at the experimental device. In natural situations, bees may forage on a food source represented by an unlimited number of available flowers rather than over a limited system like our 12-flower simulator, an apparatus impasing constant revisits of flowers. In the former case, abandonment or switching from one flower patch to another could be favoured by the finding of a negative mark signaling a recently inspected zone. Thus, this would result in shortest visit times to a patch.

The efficiency of foraging bees in both experimental situations (with the air extractor off and on respectively) could be analysed taking in account the sucrose flow provided (2.04 µl/min) and the number of effective inspections which varied from one visit to another. With the air extractor off, as bees made 124.55 inspections during 16.59 min, the mean volume of sucrose solution available per inspection was 0.27 µl. When the air extractor was activated, the mean volume available was slightly reduced to 0.25 µl. Thus, other aspects of foraging behaviour need to be considered in discussing the impact of this scent-marking strategy on the foraging efficiency.

We should have in mind that a bee forager in the field may profit from natural patches of flowers not limited to those she is visiting. That is, the exploited patch could be surrounded by many available flowers. The negative scent-mark would signal a visited place, allowing a shift to other, less visited, nearby places. This allows an increase in efficiency in terms of sugar/time while foraging in the field.

In our device, the effect of the negative mark on the foraging efficiency can be calculated by considering the increase of inspections to unrewarding flowers occurring with the air extractor on. With the air extractor on, Events 0 continued to occur (Table 3). Of the 170 (163) inspections performed in that experimental situation, 10 (10) (6%) were to recently visited flowers not yet replenished with nectar which might be rejected, as actually occurred in the visits with the air extractor off.

In addition, our results suggest that another component also contributes to the foraging economy, namely the foraging-movement pattern in which immediate return to the flower just visited occurs with low frequency (Event 0, Table 3). Thus, bees adjusted their movement pattern in such a way that they seldom returned immediately to an already emptied flower. It is worth making the point that a flower-position memory (Manning 1956) could not account for the foraging-movement pattern observed in our experimental device, due to the artificial patch being irregularly rotated as previously mentioned.

Further studies on the relationship between the repellent scent-mark and the sugar flow and on the possibility of the substance left by a particular bee being used by other bees are already under way.

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